THE LIVER REQUIREMENT OF THE FASTING ORGANISM.

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PLATE 21.

It is a truism among physiologists that the parenchymatous organs contain far more functional tissue than is required for immediate body needs. In the case of the liver, as much as three-fourths of the total parenchyma can be removed without eliciting other derangements than those occasionally ensuing through interference with the passage of the portal blood.[1] There always follows, though, a rapid regeneration from the fragment left and soon the organ has reached its old proportions. The tendency to the maintenance of these proportions is demonstrable by other means. If no ablation is done but merely a local diversion of the portal flow so that a part of the liver receives all of the blood from this source and the remainder none, the favored tissue rapidly increases in amount by cell proliferation while that deprived atrophies and disappears to a corresponding extent. The shifting of tissue thus entailed, which eventually becomes complete, is so ordered throughout that the total liver bulk at any one time approximates that of the entire normal organ. More striking still, the atrophy fails to occur, at least in any considerable degree, when a compensating hypertrophy is prevented by experimental methods.[2] The organism would seem to require just so much liver, no less and no more, and this much it preserves despite the profound circulatory derangement.

Ponfick believed that the regeneration which takes place after the removal of hepatic tissue is consequent on a functional demand or stimulus; and there is every reason to suppose that this is the true cause and the cause as well for the balanced shifting of tissue just

[1] Ponfick, E., Virchows Arch. path. Anat., 1889, cxviii, 209; 1890, cxix, 193.

[2] Rous, P., and Larimore, L. D., J. Exp. Med., 1920, xxxi, 609.

described. One need only recall the functional hypertrophy of other organs, as *e.g.* of a kidney after removal of its fellow. But granting that the stimulus is functional, that recurring body needs force the liver, so to speak, to be big, what is the nature of these needs? Can a single one of the tasks accredited to the organ be responsible in the matter of liver size, or does this result from a summation of demands? As the first step in an attempt to obtain a better understanding of the problem thus defined, we have made observations on the liver requirement during prolonged fasts,—under conditions, that is to say, which rule out all functional stimuli connected with the assimilation of food by way of the digestive tract.

Choice of Animals.

The size of the normal liver is notoriously subject to individual variation and to variation as result of the physiological incidents of daily life. The data from many instances are required for a clear demonstration on any factors affecting it. One must perforce use small animals. The herbivora are obviously unsuitable, if experiments on the effect of special diets are to be carried out. By process of exclusion the rat and mouse are come to as the species of choice; and of these the rat is obviously preferable, by reason of size and hardiness, and because of the numerous statistical data on record concerning it.

Changes in the liver of the rat take place with much greater rapidity than in large animals, as might perhaps be expected from its relatively brief life cycle and the generally swift pace of events within its body. For example, the complete shift of hepatic tissue occurring after local portal diversion as above described, which in the dog requires 6 months or more for completion, and in the rabbit, 2 months, takes in the rat only about 2 weeks, as we have recently found. Needless to say a great advantage of time is gained by the utilization of animals in which adjustments occur so quickly.

Method.

Since this paper is introductory to further work by the same method, we shall deal at some length with operative procedure, factors of error, and so forth.

Adult male white rats from the Institute breeding room have been employed almost exclusively. The weights as a rule ranged between 150 and 200 gm. For most of our purposes, they have been kept on wood shavings in individual jars, and fed the ration employed with success in breeding, namely an abundance of bread and milk each morning and of grain each afternoon with a small piece of dog biscuit every few days.

For the experiments on inanition here to be reported, glass jars equipped with a water bottle and having at the bottom a raised wire dais,—merely the cover of another, smaller jar,—were employed. The dais had a sufficiently coarse mesh for the feces to fall through. It and the jar were washed daily. When placed in metal metabolism cages, fasting rats waste quickly, presumably from loss of heat, and are liable to die out of hand, but in jars of glass and in a room kept at about 80°F. as ours was, they lose weight gradually and remain vigorous for from 7 to 12 days, depending on age and individual state. A close oversight was kept upon them all. The routine was to chloroform them while in good condition. Throughout the fasting period they drank copiously, so the factor of inspissation can scarcely have entered into the results.

Complicating Diseases.

Few intercurrent disorders occur to complicate observations upon the rat liver. The most obvious is the encysting of *Tænia crassicollis*, but this is rare in animals kept caged from birth as most of ours were. A more serious difficulty is presented by the liver enlargement associated with "giant spleen." Statistical observers have noted that rats with abnormally large spleens are prone to have abnormally large livers as well;[3] and we can corroborate their finding. The nature of the hepatolienal complex seems not to have been studied and it is highly obscure. The hepatozoon of Miller has been absent in our cases; the liver appeared normal histologically; and there were no striking microscopic changes in the spleen. Even in the fasting animal the liver remains very large (*vide* that of Rat 8, Table I). Fortunately, the disease, if such it be, was seldom encountered in our stock. All rats in which it was recognizable at autopsy were ruled from account in the experiments; yet there can be no doubt that mild forms of the disorder would have escaped notice.

Many white rats, no matter how carefully fostered, sooner or later acquire "lung disease," a chronic, spreading consolidation; and this constitutes a principal obstacle to their use, as previous workers have found. Animals in which the disease has a foothold can often be recognized for rejection by the presence of a sanguineous discharge about the nostrils, as well as by palpable râles. In the Institute stock it has been relatively infrequent and few of our experiments have been complicated by it. When just beginning, as an atelectatic consolidation of a lung lobe, usually the azygos, or as a few minute, scattered, fleshy grey nodules it does not notably affect the general condition, nor, in our experience, the liver size.

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[3] Hatai, S., *Am. J. Anat.*, 1913–14, xv, 87.

TABLE I.

Changes in the Liver Weight of the Fasting Rats of Experiment 6.

(a) Changes in the Whole Liver of Laparotomized Controls.

Rat No.	Body weight.				Liver weight.						Body condition.	Liver condition.	Remarks.
	Initial.	At operation.	Final.	Loss.	Initial (calculated).	Final. Total.	Final. Lobe mass.	Final total in per cent of. First body weight.	Final total in per cent of. Last body weight.				
	gm.	gm.	gm.	per cent	gm.	gm.	gm.						
1	180	177	133½	25.8	7.39	4.43	1.33	2.46	3.32		Rather thin.	Slightly atrophic.	No real emaciation.
2	178½	171½	128½	28.0	7.32	3.96	Not taken.	2.22	3.08		Thin.	Markedly atrophic.	
3	166	164½	108½	34.6	6.81	2.4	0.78	1.45	2.21		Extreme emaciation.	Markedly atrophic.	
4	177	169½	113½	35.9	7.26	3.42	1.12	1.93	3.01		Marked emaciation.	Moderately atrophic.	Azygos lobe of right lung in atelectatic consolidation.
5	163½	157	103	37.0	6.71	3.18	1.03	1.95	3.09		Marked emaciation.	Markedly atrophic.	Considerable atelectatic consolidation in lungs.
6	180	169	111	38.3	7.39	2.59	Not taken.	1.44	2.33		Marked emaciation.	Markedly atrophic.	
7	186	175½	144½	38.4	7.63	2.25	0.66	1.21	1.96		Marked emaciation.	Markedly atrophic.	
Average				34.0				1.81	2.71				
8	151	139	91½	39.4	6.19	3.82	1.17	2.53	4.17(!)		Marked emaciation.	Markedly atrophic.	Ruled out because of "giant spleen."

TABLE I.

Changes in the Liver Weight of the Fasting Rats of Experiment 6.

(b) Changes in the Liver Remnant of the Ablation Animals

Rat No.	Body weight.				Liver removed.	Weight of lobe mass.			Final weight in per cent of.		Body condition.	Liver condition.	Remarks.
	Initial.	At operation.	Final.	Loss.		Initial (calculated).	At operation (calculated).	Final.	First body weight.	Last body weight.			
	gm.	gm.	gm.	per cent	gm.	gm.	gm.	gm.					
9	163	160	115½	29.2	3.35	2.08	1.52	3.65	2.24	3.16	Marked emaciation.	Hypertrophic; slightly fatty.	
10	176	174½	123½	29.8	3.51	2.25	1.59	3.47	1.97	2.81	Marked emaciation.	Hypertrophic; slightly fatty.	Atelectatic consolidation of upper quarter of left lung.
11	170	162	112½	33.8	3.4	2.18	1.54	3.31	1.95	2.94	Moderate emaciation.	Hypertrophic; not fatty.	
12	150	143	94	37.4	3.02	1.92	1.37	2.57	1.71	2.73	Marked emaciation.	Hypertrophic; not fatty.	
13	180½	171	112	37.9	3.6	2.31	1.63	2.63	1.46	2.35	Very marked emaciation.	Slightly hypertrophic.	
14	166	158	96½	41.8	3.73	2.13	1.71	2.09	1.27	2.17	Extreme emaciation.	Normal.	Moribund when killed.
15	150	142	81½	45.7	3.83	1.92	1.75	1.72	1.15	2.11	Extreme emaciation.	Slightly atrophic.	Hematuria; died; autopsied prior to clotting of blood.
Average				36.5					1.68	2.61			

Explanation of Table.—The initial body weights of the table were obtained after a 6 hour fast. There was a further fast of some 18 hours prior to operation, involving a not inconsiderable further loss of weight.

The initial liver weights in the control series is calculated on the basis that 4.1 per cent of the body weight consisted of hepatic tissue; and the initial weight of the lobe mass on the further assumption that of the hepatic tissue 31.2 per cent lay in said mass.

Measurements.

The length of the rat from nose to anus has regularly been ascertained since it yields with the weight some index to the nutritive condition of the animal. Well nourished, but not fat, individuals were chosen for the work. Immediately after the final chloroforming the livers were taken out, and in consequence they were obtained in a relatively bloodless state. The point is an important one because when autopsy is delayed but a few minutes, much blood remains in the hepatic tissue after excision despite the circumstance that clotting has not yet occurred. In the lack of other, more satisfactory criteria, the gross weight of the liver was of necessity taken as the indicator of the true amount of the organ present in the body, meaning by true amount that of parenchyma. It is known that liver tissue may on occasion become transiently swollen with glycogen or fat, as further that its protein content may be altered by the feeding of protein.[4] During digestion the organ may be 12 per cent larger than between whiles.[5] In order to lessen the error from these sources in establishing a normal weight for the liver, the healthy rats to be examined were deprived of their usual ration on the day on which they were to be killed, and were sacrificed about 6 hours after this feeding would ordinarily have been given. The stomach was regularly found empty at this time and the intestine nearly so; while the liver was rather sharp edged and flabby with little if any fat visible histologically and almost no glycogen. The gross body weight now closely approached what Jackson[6] calls the net body weight (gross body weight less that of the intestinal contents). Little was to be gained by waiting longer, since the intestinal contents underwent only a negligible decrease in bulk during a further fast of 18 to 24 hours. Throughout inanition, as Jackson has found, the intestines retain material to the amount of some 2 to 3 per cent of the gross body weight. We have not attempted to determine the figure in each of our animals but have been content with the gross weight.

Effects of Inanition on the Liver.

In human beings and lesser animals receiving water but no food, the liver undergoes a remarkable diminution in size. Only the adipose tissue loses more in weight. The differing loss by various organs has given ground for the belief that those suffer least which are essential to the body economy, as, for instance the heart.[7] Voluntary muscles that are frequently used waste less than those unem-

[4] Tichmeneff, N., *Biochem. Z.*, 1914, lix, 326.

[5] Howell, W. H., Text-book of physiology, Philadelphia and London, 6th edition, 1917.

[6] Jackson, C. M., *Am. J. Anat.*, 1915, xviii, 75.

[7] Aschoff, L., *Path. Anat.*, 1911, i, 41.

ployed. Manassein[8] holds, in accordance with these facts, that the liver changes result from a lessening functional demand which is consequent on the fasting. Paschutin,[9] on the other hand, has propounded the view that the various tissues lose during inanition in proportion as they are themselves available as food. The difference in opinion brings up a crucial point as regards the relation of the liver to the body needs. Has this portal organ any great uses during inanition? Will not an inconsiderable fraction of it suffice under such circumstances? It has seemed to us possible to obtain an answer to these questions by a simple experiment. By operation one can summarily reduce the body content of hepatic tissue to less than the amount persisting in advanced inanition of the intact animal. When now food is withdrawn from rats treated in this way, what will happen? If the reduction in size of the liver that occurs ordinarily during inanition is the result of a utilization of the organ as food then one might expect a dwindling of the fragment left at operation; but if the liver size is determined by functional needs then perhaps the fragment will hypertrophy, despite the general food lack, to meet the necessities of the fasting body.

The liver of the rat, as of the cat, dog, and rabbit consists of two masses of unequal bulk each with its own vessels and ducts, connected by an inconsiderable isthmus of parenchyma. In the rat the larger mass, or "main liver," lying between stomach and diaphragm, contains, as we have found in twelve normal animals, an average of 68.8 per cent of the total hepatic tissue. The operative removal of this mass is well borne. The average for the smaller moiety, or "lobe mass," lying above the right kidney, is, as would follow for the figure given above, 31.2 per cent and the greatest variations noted were 25.7 and 34.7 per cent, respectively. The individual figures in most instances came remarkably close to the average. According to Jackson[6] rats in extreme inanition still have some 42 per cent of liver tissue left. There is reason to suppose the figure to be an unduly low one. For the normal weights on which Jackson's calculation of the amount of change was based were those of organs from animals well fed to the time of death, and having for that reason an hepatic parenchyma containing much extrinsic material. In such rats the livers constituted 4.3 to 4.5 per cent of the total body weight, whereas

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[8] Manassein, W., Dissertation, St. Petersburg, 1869, abstracted by Mühlmann, M., *Centr. allg. Path. u. path. Anat.*, 1899, x, 160.

[9] Paschutin, W., *Vorlesungen über allg. Path.*, pt. 2, St. Petersburg, 1881, cited by Mühlmann, M., *Centr. allg. Path. u. path. Anat.*, 1899, x, 160.

in ours they averaged only 4.1 per cent. Whatever the true figure, the important point is that removal of the main liver, which can be accomplished by operation, entails a reduction in the amount of hepatic tissue well below that obtaining in most instances after a prolonged fast.

Removal of the Main Liver.

Under ether anesthesia, with aseptic conditions, and the rat on an electrically heated pad, the abdominal wall is slit through from the junction of the ensiform cartilage and manubrium to well below the anterior border of the liver, a distance of 3 to 5 cm. The exposed ensiform springs outward of itself, yielding access to the liver high on the diaphragmatic surface; and the incision is held open with an eye speculum. The filmy, tough membrane which loosely connects the under surface of the main liver with the papillary lobe is torn through, a small sponge is so placed as to hold the two widely apart, and a thin edged, flat, aneurism needle is gently thrust between the portal trunk to the main liver and the underlying vena cava. Several attempts will often have to be made before it passes. The eye of the needle is now threaded with very fine silk (No. 1 surgical) and the portal trunk with the accompanying artery and bile duct are all included in one ligature.

The remaining steps in the removal of the main liver are simple. The suspensory ligaments are torn through, freeing it save for the caval and gastrohepatic connections; a thread of strong, thin silk (No. 2 surgical) is carried around the mass between it and the diaphragm; the gastrohepatic omentum is picked up with curved mosquito forceps just above the portal tie and severed with scissors; and by the pull of the forceps, the mass is everted while the thread already in place at the junction of the hepatic and caval veins is tied down. If slight traction be made upon the thread as the knot is laid, it will come to include only the veins first mentioned. The liver mass can now be cut away at leisure, leaving a neat small stump. Closure of the abdominal wall is done in two layers, with No. 1 silk; and no dressing is applied. Healing by first intention is the rule. The whole operation can ordinarily be completed in 12 to 15 minutes. Passive congestion does not follow; and except for intercurrent disease there would be almost no mortality. About one animal in ten is lost from this latter cause.

The control rats were treated in every way like the ablation animals except that a laparotomy only was performed on them and the opening held apart with a speculum during the time ordinarily required for removal of the main liver.

Changes after the Ablation in Fed Rats.

In well fed rats the lobe mass increases in size with astonishing rapidity after removal of the main liver. Ponfick's[10] classical description of the course of events in rabbits so treated renders super-

[10] Ponfick, E., *Virchows Arch. path. Anat.*, 1895, cxxxviii, suppl. 81.

fluous a detailed account of the changes. The increase in tissue comes about by an interposition, to use Ponfick's term, of new cells here and there throughout the lobuli already existing. We find that in rats still young but sexually mature and weighing 130 to 150 gm., the lobe mass reaches the size of the entire original liver in from 9 to 12 days; whereas in old animals of 300 gm. or more it has attained only two-thirds this bulk after the longer of the periods mentioned. Many observations would be required for exact knowledge of the rate of regeneration at different ages, and fortunately it is not essential for present purposes.

Changes in Fasting Animals.

The effects of fasting were observed in six experiments, each with appropriate controls. Forty-five animals in all were successfully studied. In the early work the animals fasted for only 6 hours prior to removal of the main liver, and for this reason the influence of residual food in the intestine cannot be wholly ruled out, as it was later by a preliminary fast of 24 to 48 hours. Furthermore, the controls were not operated upon. The animals of a first series were kept on shavings, some of which they ate and their feces as well, while in another they were confined in metal metabolism cages and lost weight with great rapidity. But the results of these imperfect experiments were identical with those of the better ones and possess a corroboratory value.

Profound differences were regularly to be noted between the liver changes in the ablation rats and those in the controls killed at the same stage of advanced inanition. In the latter the atrophy of the entire liver, described by all previous observers, was well marked, whereas in the former the fragment of hepatic tissue left behind at operation had in most cases undergone an indubitable hypertrophy.

According to an extensive literature[11] the liver of inanition is remarkably small and flabby, and sharp edged, tough because of the great relative diminution in parenchyma, and suffused with blood owing to a widening of the intralobular capillaries consequent on

[11] Aschoff, L., *Path. Anat.*, 1911, i, 41. Morpurgo, B., *Beitr. path. Anat.*, 1889, iv, 313. Mühlmann, M., *Centr. allg. Path. u. path. Anat.*, 1899, x, 160.

atrophy of the liver cords (Fig. 3). Intralobular hemorrhages are said to occur but these we have not seen. All of the parenchymal elements do not suffer equally, the changes being much more marked toward the center of the lobule, the cell cords tapering from a relatively broad base of approximately normal elements next the interlobular connective tissue to a narrow acuminate column of tiny entities having a mere rim of protoplasm about a much diminished dark nucleus. The average diameter of the cells of entire lobules may come to little more than half the normal, while that of the nucleus may be about three-tenths of it,[12] figures which imply extreme changes in the elements near the central veins. Here the widening of the capillaries gives the picture a superficial similarity to that of chronic passive congestion. When the vessels are cut and the blood escapes the liver falls in on itself, so to speak.

All these changes were present in our control rats killed after 9 to 11 days of fasting, when 25 to 40 per cent of the body weight had been lost. But diametrically different, as already stated, were the findings in the ablation animals, although in them the loss of body weight was on the whole more pronounced as would follow from the mechanical removal of several grams of hepatic tissue, part of which might have been utilized secondarily, as food. In these emaciated creatures the small liver mass left behind at operation was in most instances enlarged and rounded, friable because of a disproportionate increase in its parenchyma, and, when the vessels were severed, rendered almost bloodless (Fig. 2) by the pressure of the growing tissue upon the capillaries. Microscopically, the changes were those of a genuine hypertrophy in Ponfick's sense, and similar in all ways to those occurring after a like ablation in well fed animals except that they were less marked. The individual lobuli were far larger than those in the main livers removed at operation from the same rats, owing to the increased length of the cell cords, which latter contained many more cells than is normal and these undiminished in size (Figs. 1 and 2). Globules of fat were not infrequently present in the parenchyma near the central vein, as in some well fed animals, but usually they were absent and the hepatic tissue appeared normal save for the changes incident to its proliferation.

[12] Lasarew, N., *Russ. Arch. Path.*, 1897, iii, 399, cited by Mühlmann, M., *Centr. allg. Path. u. path. Anat.*, 1899, x, 160.

The Influence of Age.

Jackson[13] has compared the effects of inanition on adult and young rats, and has pointed out that in the latter a growth impulse must be recognized as influencing the size of the liver. Our animals were well beyond the age at which this impulse becomes negligible, but, owing to the unusual conditions introduced by the ablation, experiments seemed called for to determine whether the hypertrophy of the lobe mass during inanition would take place in old animals. It does take place. In two old rats of 290 and 320 gm., subjected to an ablation, the same changes occurred as in ordinary adults (Figs. 1 and 2).

The Ultimate Amount of Liver.

As data accumulated, a significant fact made itself evident. Not only did the lobe mass hypertrophy in the ablation rats but its ultimate weight closely approximated that reached by the entire liver of the controls through a process of atrophy. The final experiments were framed with special attention to this point. In order to rule out completely the factor of residual food as a stimulus to hypertrophy the animals were subjected to a preliminary fast of from 24 to 48 hours in jars with a dais. So rapid are body processes in the rat that the main livers removed after this brief period showed plainly the changes characteristic of early inanition (Fig. 1).

The results of one experiment will be given *in extenso* (Table I, Text-fig. 1). The data from all the closely controlled material find expression in Text-fig. 2.

Experiment 6.—Fifteen well nourished male adult rats, closely paired in weight and length, were operated upon after a 24 hour fast. Their weight just prior to anesthesia is the "first weight" of Table I. The main livers were removed from seven of them; while in the case of the others a laparotomy only was performed. Despite continued fasting, all made a good recovery. One ablation rat (No. 15) died of inanition 7 days after the operation,—or after 8 days in all, without food. It was autopsied practically at once. The findings offer an extreme example of the changes manifest in the others. These were killed on the same day while still in good condition. The abdominal wounds were healing with extreme slowness though by first intention.

[13] Jackson, C. M., *J. Exp. Zool.*, 1915, xix, 99.
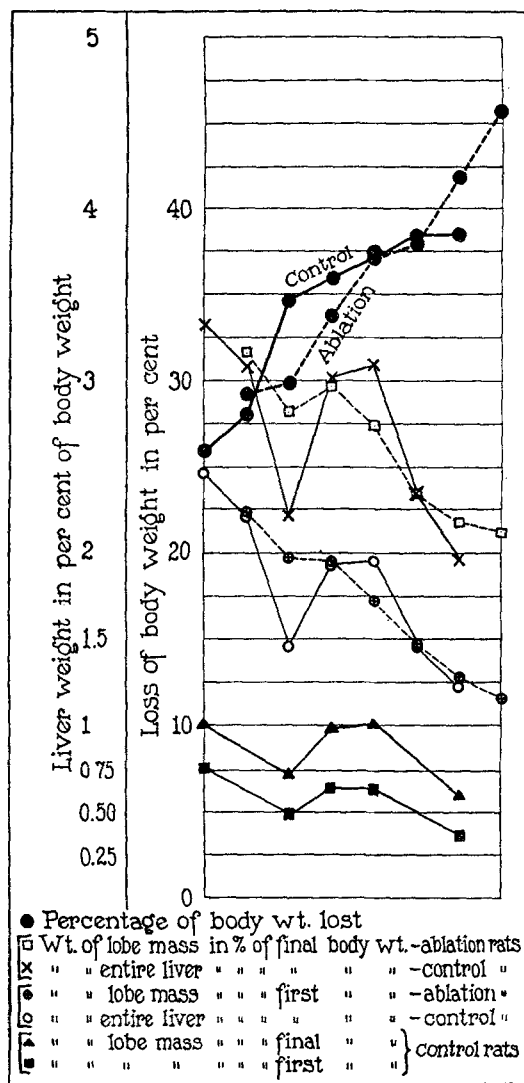
Each solid black dot records the loss in body weight of a rat. On the same perpendicular there are placed other symbols recording data on the liver weight of the animal, as expressed in terms of the body weight. In order to distinguish between the material of the control and ablation rats, the symbols pertaining to each series have been joined up with lines.

TEXT-FIG. 1. The duplication occurring in the liver weights of fasting ablation animals and controls (Experiment 1).

According to Jackson, white rats may be expected to tolerate an average loss through fasting of about 40 per cent of the gross body weight. The animals of the present experiment lost between 25.8 and 45.7 per cent of an approximately *net* body weight, which is to
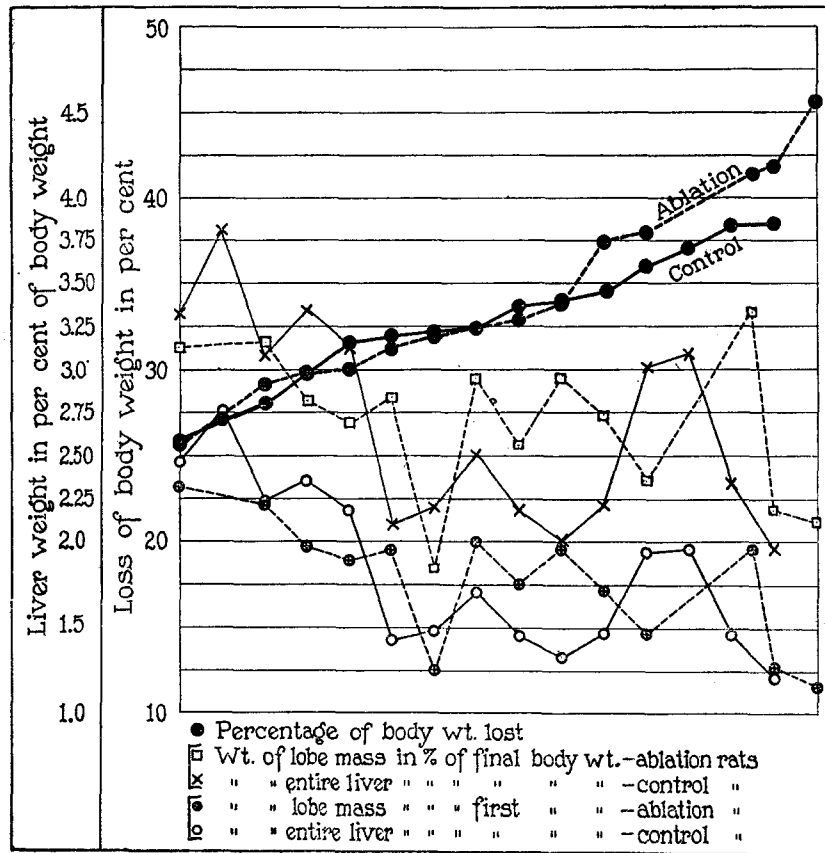
TEXT-FIG. 2. The duplication occurring in the liver weights of fasting ablation animals and controls (general results).

say that some of them were at the extreme of inanition. Those of the ablation series were in general most affected as in the previous series. To cope with the difference thus introduced into the data these latter have been grouped in the chart with special reference to

the body loss, the findings in each ablation animal being superimposed upon those of a control that had lost to the same amount. But since the nutritive condition of the animals at the end of the experiment was not always accurately expressed by the percentage loss in tissue, descriptive terms have been employed as well in the table. In the "extremely thin" animals only traces of fat could be seen anywhere in the gross at autopsy; in those that were "very thin" there still existed a little fat in the omentum and retroperitoneal tissue but none in the subcutaneous tissue; while in the "thin" creatures there was some fat at this latter situation and more within the peritoneal cavity. The one rat that was merely "rather thin" had still much intraabdominal fat.

In the analysis of the findings one control (No. 8) was ruled out because of the "giant spleen" with which an abnormally large liver is associated. The animal had lost more weight (39.4 per cent) than any other, yet the liver had undergone almost none of the usual atrophy and histologically the tissue appeared normal. The instance well illustrates the importance of the hepatolienal complex as affecting work with the rat liver.

For purposes of comparison the weights of the entire liver of the controls and of the lobe mass of the ablation series have been expressed in percentages both of the initial body weight and of that at the time of death. No inconsiderable proportion of the bulk of the atrophic control livers was made up of blood contained in the sinuses between the atrophic parenchymal columns, whereas in the hypertrophic lobe masses of the experimental animals this element was almost lacking, as already mentioned.

The liver of one of the control rats losing greatly (No. 7) had so far atrophied that its final weight was, on calculation, about the same as that of the lobe mass alone at the time of operation. It now constituted 1.21 per cent of the initial body weight, whereas the lobe mass at the beginning may be supposed to have come to about 1.28 per cent, allowing that this mass constituted 31.2 per cent of the tissue of the original whole liver and that the latter amounted to 4.1 per cent of the body total. In two other cases (Nos. 3 and 6), there was almost as great a degree of atrophy. The actual diminution in parenchyma was even greater than the figures indicate because of the suffusion of the atrophic tissue with blood.

In the ablation animals corresponding with the more extreme of these instances, there was no need of hypertrophy of the lobe mass for it to be as large as the total liver of the controls. The mass need only hold to its original bulk, or, when this was exceptionally great, it might even atrophy a bit. And this was precisely what happened. In ablation Rat 14 there occurred, as the figures show, what may be termed a permissible slight atrophy, while in No. 13 the tissue did not alter from the normal. These cases stand in an enlightening contrast to others in which an amount of liver tissue equivalent to that present in the controls came into being through an active hypertrophy. In three ablation rats the bulk of the lobe mass must have practically doubled during the short period after the operation. Its final weight equalled that of the main liver that had been removed from the same individuals, and was several times that of the lobe mass in the controls, which was extremely small as the chart shows.

Nature of the Changes.

The first question in connection with these findings is whether they may not be accidental. The enlargement of the lobe mass following the ablation might perhaps be set down as inevitable to the increased flow of portal blood, did not atrophy or no change at all sometimes occur instead. In many species there takes place early in starvation an active transfer of body fat to the hepatic cells which show large or small globules of it, especially at the periphery of the lobuli. In the rat, according to Mottram[14] this transfer is lacking, at least to any noteworthy extent; and under the conditions of extreme inanition with which we are here concerned, it is negligible even in the species mentioned. In our rat livers, fat was visible only in the largest of the hypertrophied lobe masses wherein some globules were to be seen. These made up but a negligible portion of the total bulk and were central in situation instead of peripheral, as are those of fat transferred ordinarily during a fast.[15]

Analyses have shown that the liver tissue in instances of death from inanition yields about 10 per cent of fat, which is not demon-

[14] Mottram, V. H., *J. Physiol.*, 1909, xxxviii, 281.

[15] Statkewitsch, P., *Arch. exp. Path.*, 1894, xxxiii, 415.

strable histologically.[16] The atrophic and hypertrophic tissues of the present experiments might well be found to differ in such respect. Yet assuming this to be the case, one still could not be certain whether it was not perhaps the expression of a difference in the essential make-up of the liver protoplasm in the contrasted series of animals, rather than one in amount of extraneous material there deposited. That there was a difference in the amount of parenchymal protoplasm present in a like weight of the atropic and hypertrophic liver tissue is certain. The proportion was less in the former, owing to the suffusion of it with blood. Furthermore, the quantitative relationships between cytoplasm and nucleus were markedly abnormal in the atrophic control livers, as others before us have noted,[12] whereas there was in this respect but little that was unusual in the cells of the hypertrophic lobe masses. If it is impossible to state of two like bulks of cells of the same appearance and apparent condition that they have identical functional capacities, how much more vain would it be to assert this of unlike bulks with cells similar only in their derivation as in the present case!

The fact remains, and remains significant, that there was a close approximation in weight between the liver masses in our two sets of animals and that this occurred, in the one case by a simple atrophy, in the other by what may be termed with equal reason simple hypertrophy. And not only this, but when an hypertrophy of the lobe mass was rendered unnecessary by the circumstances of the case, atrophy of it or no change at all was what occurred, with result that the mass still duplicated in weight the main liver of the appropriate control. This is strong evidence that both bulks of tissue, whatever their incidental difference in parenchymal content and in appearance of the cells, constitute expressions of a response to body demands of like magnitude. One may conceive of both parenchymas, though so different histologically, as having to perform and performing the same amount of work.

Significance of the Findings.

There are precedents in the behavior of other organs for the hyper-

[16] Rosenfeld, G., *Ergebn. Physiol., 1. abt.,* 1903, ii, 50, 86.

trophy of the liver that occurs during starvation. McCarrison[17] has shown that in fasting pigeons the adrenal glands enlarge, and Jackson's figures indicate that they do so in rats that are fasted. Very recently Vincent and Hollenberg[18] have presented data which show that in this latter species a nearly fourfold increase in the weight of the adrenals takes place during an inanition period of 10 to 13 days, and an almost threefold increase in the thyroid weight, which is accompanied by a disappearance of the colloid material. These findings suffice to show that the liver changes are not unique in character.

Mention has already been made of the alternative explanations which have been advanced to account for the pronounced hepatic atrophy in fasting individuals. According to one set of workers the organ is primarily used up as food, while others hold that it decreases in amount because of the decrease in functional demands. Needless to say, the results of our experiments are wholly in keeping with this latter view. The explanation that the liver tissue necessarily undergoes destruction for food is rendered untenable not alone by our data but by the demonstrated hypertrophy of certain other organs during inanition, in contrast to the atrophy then generally occurring. That there exists a "padding" of glycogen and fat in the liver, which is utilized as food early in inanition no one will deny, nor that somewhat later, fat mobilized from elsewhere in the body may be deposited in the organ only to be removed again; while that liver tissue disappearing at late stages in inanition is secondarily utilized as food can scarcely be doubted. In this connection it may be remembered that our control animals emaciated less rapidly than those from which liver tissue had been removed (Table I). Yet with all said, there is excellent reason to conclude that the primary cause for the disappearance of liver tissue during inanition lies in a lessened functional need.

Extent of the Changes.

In discussing the extent of the liver changes we shall make use of the data of Experiment 6 only, inasmuch as this experiment was the most carefully carried out and controlled. The diminution in hepatic

[17] McCarrison, R., Indian J. Med. Research, 1918–19, vi, 275.

[18] Vincent, S., and Hollenberg, M. S., J. Physiol., 1920–21, liv, p. lxix.

tissue in our control animals amounted on the average to 53.7 per cent. In Jackson's rats with inanition comparable in degree it came to 58 per cent, but his normal average for the liver, which formed the basis of calculation, was higher than ours, owing to the circumstance that it had been obtained by observations on the organs of full fed rats instead of ones subjected as ours were to a brief fast. The amount of liver tissue ultimately present in our rats varied with the loss of body weight. It ranged in the case of the controls from 1.21 to 2.46 per cent of the initial body weight, with an average of 1.81 per cent instead of the 4.1 per cent that is normal. In the experimental animals the ultimate amount of liver tissue was smaller, from 1.15 to 2.24 per cent of the initial body weight, with an average of 1.68 per cent. This is below the average for the controls but the animals were in general thinner. The chart demonstrates that under like individual conditions the liver weight was the same for both series.

The Liver Requirement During Inanition.

If it be assumed, as the facts seem to warrant, that the liver weight late in inanition is determined by the functional needs, then the data of our experiment should give some indication of the dimension of these needs. The normal liver makes up 4.1 per cent of the net body weight. But of this weight some 10 per cent consists of what Jackson[6] terms the ligamentous skeleton, structures which alter but little in the adult even during inanition and which must make an almost negligible day-to-day demand on the hepatic tissue. The normal percentage relationship of the liver weight to the net body weight minus that of this skeleton, that is to say the relation of the liver weight to the soft body weight comes to 4.56 per cent. In our animals the final liver weight as expressed in that of the final body weight was for the controls 2.71 per cent and for the operated rats 2.61 per cent—the general average being 2.66 per cent. But according to Jackson, the ligamentous skeleton makes up some 15 per cent of the final body weight. When allowance has been made for this the liver percentage as expressed in the weight of the soft body comes to 3.2 per cent, or to about three-fourths of the normal. Of the missing

quarter some part assuredly consisted of "padding,"—fat and gly-
cogen on temporary deposit in the organ. Altogether the findings
indicate that during inanition there is large need for a liver.

Possible Nature of the Requirement.

What is the nature of this need? The hepatic tissue is known to
be busily concerned under normal conditions with the tasks of inter-
mediate metabolism. During inanition the intermediate metab-
olism still goes on, though it is true that the substances worked upon
are no longer derived from the digestive tract but mobilized from the
tissues.[19] Some of them, fat and to a less extent glycogen,[20] are
temporarily laid down in the hepatic cells. In the case of fat, there
may be wave-like fluctuations in the amount present from day to day,
as inanition progresses.[14] The fact that under circumstances which
involve a ruling out of ordinary digestive processes the liver still
remains large, relatively speaking, does not necessarily mean that it
is not normally a portal organ, receiving on the blood from the diges-
tive tract the functional impacts, so to speak, which are responsible
for its size. For impacts of the same sort still come to it during
inanition but from a different source and direction.

After an Eck fistula has been produced the liver undergoes a per-
manent reduction in size of about one-fifth.[21] If functional demands
determine liver size this can only mean that the organ has less to do.
For the alternative explanation, that enough blood cannot be supplied
through the hepatic artery to nourish a larger organ, is untenable.
There are too many instances in which, on occasions demanding
functional hypertrophy, the blood supply undergoes an increase *pari
passu* with the tissue increase, as *e.g.*, during the compensatory
adjustment after removal of a kidney. In the case of the Eck fistula
liver, one may suppose that some share of the normal hepatic task
is, owing to the changed circulatory arrangement, performed else-
where in the body. For many of the normal activities of the liver
are not unique with the organ but are shared by others to a less extent.

[19] Lusk, G., Science of nutrition, Philadelphia, 3rd edition, 1917.

[20] Junkersdorf, P., *Arch. ges. Physiol.*, 1921, clxxxvi, 238. Laquer, F., *Klin.
Woch.*, 1922, i, 822.

[21] Whipple, G. H., and Hooper, C. W., *Am. J. Physiol.*, 1916–17, xlii, 544.

During inanition this is doubtless still the case. And while the portal flow still goes on at such a time, the portal blood cannot but differ much less from the systemic than usual. It no longer carries with it so many special functional opportunities. The failure of the liver of fasting animals to be quite as large, relatively speaking, as the ordinary, may be consequent on this changed condition of affairs, whereby the liver loses in opportunity over the other tissues.

The character of the demands made upon the liver cannot but differ much in early and late inanition. So long as the fat reserves of the body hold out, the metabolic needs are supplied almost entirely from this source.[19] It is unnecessary to refer again to the evidence of fat mobilization and transport to the liver. Later when the body fat is exhausted, protein is called upon, and now emaciation proceeds with a special rapidity owing to the amount of tissue used up for the needs of each day. In our ablation animals it was noteworthy that whereas those killed while still in possession of some fat, though much emaciated, had a hypertrophic lobe mass, others fasting only for the same time but with a practically complete exhaustion of the fat deposit possessed an hepatic tissue that was either atrophic or unchanged. These latter had begun to use up tissue protein at an unprecedented rate; yet the livers were small. The question arises whether this was because the demands on the organ were less when protein was burnt or because the needs of the body for food took precedence over those for a large liver. Certain facts indicate that the explanation first mentioned may be the correct one. The body is able to handle proteins elsewhere than in the liver. Atrophy is not inevitable to extreme inanition, as the hypertrophy of the adrenal and thyroid sufficiently shows. While at the time when death occurs from inanition abundant food for the cells may still be present in the body.

SUMMARY.

In rats fasted after the removal of about seven-tenths of the liver the remaining fragment undergoes ordinarily a marked simple hypertrophy, and attains the weight reached through a process of simple atrophy by the entire liver of fasting controls. Under circumstances of exceptionally severe inanition, the hypertrophy may not occur, the

fragment remaining unchanged or even undergoing a slight atrophy. But since in comparable controls the hepatic atrophy is extreme, the duplication in the liver weights still manifests itself.

It is difficult to avoid the conclusion that the liver changes which take place in fasting animal are essentially conditioned on functional demands made upon the organ. Yet great as is the hepatic atrophy then occurring, this does not necessarily mean that these demands are much lessened during inanition. For a comparison of the liver weight with that of the soft body (net body weight less the weight of the ligamentous skeleton) brings out the fact that the organ is, relatively speaking, three-fourths as large at extreme inanition as in the well nourished individual. Of the missing fourth a part may be foodstuffs on temporary deposit. And the rest is perhaps to be accounted for by the existence of special conditions during inanition which favor the vicarious assumption of a part of the usual liver work by the other tissues.

While the findings show that the liver size is essentially dependent on functional demands, they do not enable one to say whether the special demands in question come normally to the organ by way of the portal stream.

EXPLANATION OF PLATE 21.

Figs. 1, 2, and 3. To show the histological character of the liver changes in an ablation rat and its appropriate control. All of the figures have the same magnification, 150 diameters. Eosin and methylene blue.

Fig. 1. Section of the main liver removed after a 48 hour fast from a rat of 290 gm.

Fig. 2. Section of the lobe mass of the same animal after 7 days more without food.

Fig. 3. Section of the lobe mass of the fasting control animal which was submitted merely to a laparotomy. The initial weight of this rat was 302 gm.

The control lost 36.7 per cent in body weight and the ablation rat 37.4 per cent. The lobe mass of the latter weighed 4.64 gm. at autopsy, whereas the entire liver of the control came to only 4.78 gm.

PLATE 21.
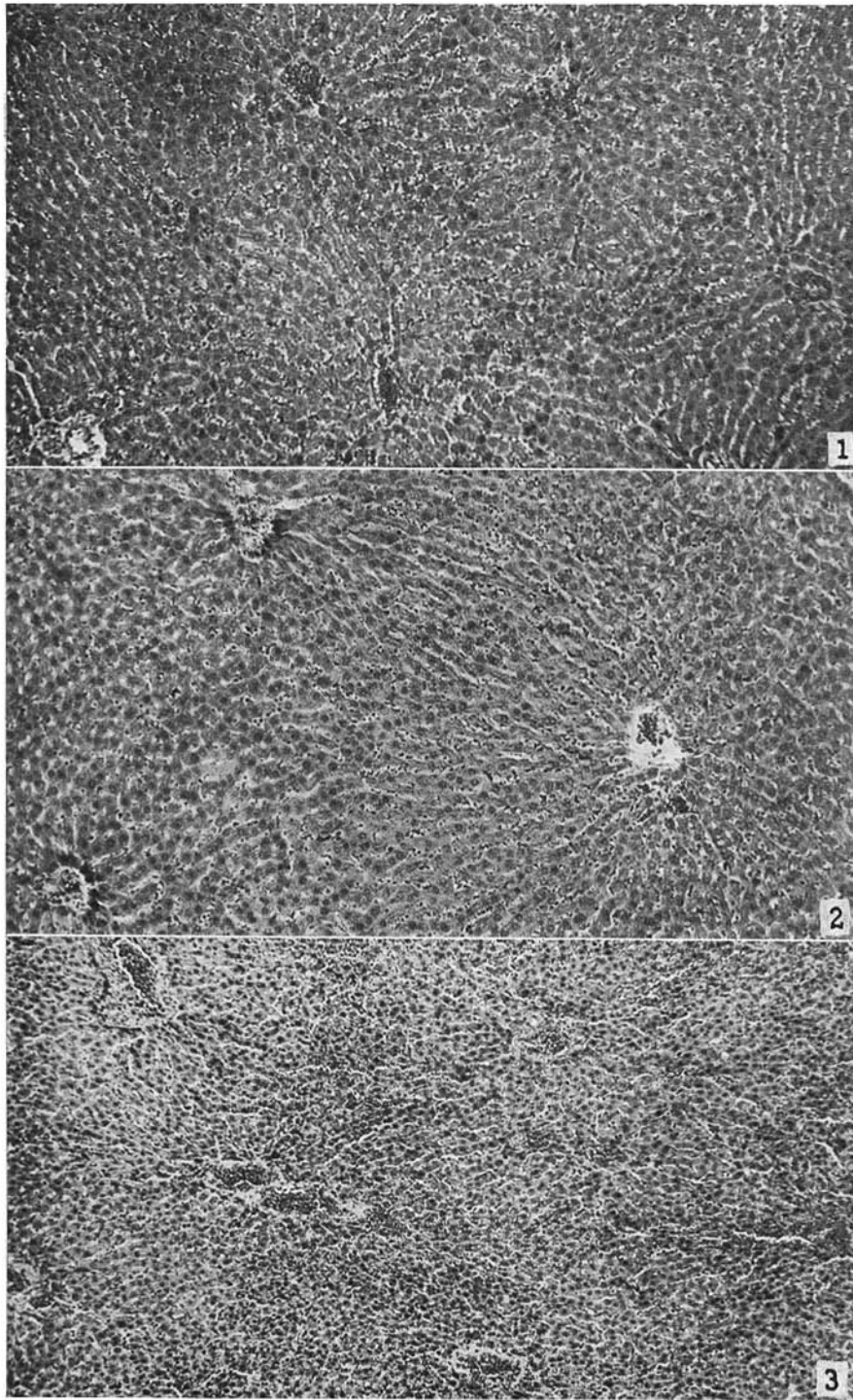
(Rous and McMaster: Fasting organism.)